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ABSTRACT Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is an important quarantine pest of nurseries. Nursery plant movement from *P. japonica*-infested regions is regulated by the U.S. Domestic Japanese Beetle Harmonization Plan (DJHP), which classifies states by risk categories. Treatments for category 2 states include preharvest soil surface treatment of nursery plants grown in field soil using Discus SC, Marathon (1G or 60 WP), or Flagship (0.22G or 25 WG). In this study, Discus, Marathon 60 WP, or Flagship 0.22G DJHP standards were compared with labeled rates of non–DJHP-approved insecticides, including neonicotinoids clothianidin (Arena 50WDG), generic imidacloprid (Quali-Pro Imidacloprid 2 F T&O Insecticide, Mallet 2 F T&O Insecticide, and Lada 2 F Insecticide), and imidacloprid + bifenthrin (Allectus SC), as well as the anthranilic diamide, chlorantraniliprole (Acelepryn Insecticide). Arena provided 100% *P. japonica* control in May, June, and July over four test years, but had one larva recovered during August in two of those 4 yr. Acelepryn did not provide DJHP-acceptable *P. japonica* control. During July, Allectus provided 100% *P. japonica* control in three of four test years, but had four larvae in one test year. Other treatments tested only during July, which provided 100% *P. japonica* control, included Discus (five tests); Marathon, Quali-Pro, and Mallet (two tests); and Lada and Flagship (one test). Generic imidacloprid 2 F formulations were equivalent in *P. japonica* control to DJHP-approved insecticides. Insecticides generally performed poorly on other scarabs or curculionid larvae. The study supports Arena, Allectus, and generic imidacloprid 2 F products as suitable candidates for the DJHP.

KEY WORDS *Popillia japonica*, neonicotinoid, harmonization plan, scarab, insecticide

Japanese beetle (*Popillia japonica* Newman) (Coleoptera: Scarabaeidae) remains an important agricultural and horticultural pest in the United States almost 100 yr after its introduction (Potter and Held 2002, Jackson and Klein 2006). The beetle has a broad host range that includes many important agricultural and ornamental plants (Fleming 1972a, Held 2004, Szendrei and Isaacs 2006, Tiddens and Cloyd 2006). U.S. Department of Agriculture–Animal Plant Health Inspection Service (USDA–APHIS) (2007) estimated about US$694 million/yr in costs associated with Japanese beetle, but these figures may be an underestimate in current dollar values and with the beetle's continued westward range expansion.

About three-fourths of the Japanese beetle life cycle occurs within the soil (Fleming 1972a). Therefore, nursery plants shipped with roots and soil are an extremely important and problematic route for accidental movement of Japanese beetle (Fleming 1972b, National Planting Board [NPB] 2011). Scarab pests are extremely difficult to eradicate once established (Jackson and Klein 2006), which adds to the threat potential if introduced into noninfested central and western states. It is likely most of the major pest scarab species in the United States, including Japanese beetle, oriental beetle (*Anomala orientalis* [Waterhouse]), European chafer (*Rhizotrogus majalis* [Razoumowsky]), and Asiatic garden beetle (*Maladera castanea* [Arrow]), were introduced with soil of nursery plants (Jackson and Klein 2006).

Shipment of nursery plants grown in soilless media substrates or field soil from Japanese beetle-infested states is presently regulated by the U.S. Domestic Japanese Beetle Harmonization Plan (DJHP) (NPB 2011). The DJHP is an agreement between participating states with the objective of developing standard Japanese beetle management protocols to reduce the risk of moving the pest, while maintaining orderly marketing of affected regulated articles like nursery

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stock (NPB 2011). Under the DJHP, states are categorized based on their current Japanese beetle infestation status and the likely impact should the beetle establish in the state. Treatment options vary depending on the state's category and risk status. Category 3 states are located mostly in the eastern United States and are considered infested with Japanese beetle. Shipments of container or field-grown nursery stock are permitted between category 3 states without treatment for Japanese beetle. Category 4 states are historically not known to be infested with Japanese beetle and are believed to be unsuitable for beetle establishment; therefore, category 4 states also have no regulatory requirements for treating nursery stock to eliminate Japanese beetle. The remaining state categories require some type of treatment to certify nursery stock against Japanese beetle. Category 1 states are mostly west of the Rocky Mountains. The agricultural impact of Japanese beetle establishment in category 1 states is anticipated to be moderate to high, and the beetle is not expected to naturally disperse to these regions; therefore, category 1 states manage the beetle as a quarantine pest with zero tolerance. Category 2 states are mostly located west of the Mississippi River in the central United States and regulate Japanese beetle as a nonquarantine pest. Japanese beetle is predicted to naturally expand its range into category 2 states, where the impact is likely to be low to moderate (NPB 2011). Some category 2 states are also partially infested with Japanese beetle.

Treatment requirements for category 1 states are more stringent than category 2 states. Category 1 states only allow entry of "plants grown in nonclay soil" from Japanese beetle-infested states (NPB 2011), which typifies most field soils. Insecticide adsorption, persistence, and movement in field soil are affected significantly by the inorganic minerals that form clay particles, as well as soil organic matter (Sheng et al. 2001, Anhalt et al. 2008). Field soils also differ considerably from nursery container substrates like pine bark in physiochemical properties (Simmons and Derr 2007), and many of these soil properties reduce Japanese beetle treatment efficacy depending on the chemical components of the insecticide (Cowles and Villani 1994). Category 2 states allow any treatment approved for category 1 states. In addition, category 2 states will accept nursery plants grown in clay-containing field soils or container substrates. Category 2 states also accept plants with large soil volumes (81-cm-diameter plants) than category 1 states (30 cm diameter).

There are presently two approved methods to ship nursery plants grown in clay-containing field soils from category 3 to category 2 states. The first method is a postharvest root ball immersion in chlorpyrifos or bifenthrin, and the second is a preharvest soil surface insecticide application between May and July banded 15 cm wider than the root ball diameter being harvested (Mannion et al. 2000, 2001; Oliver et al. 2007, 2009; NPB 2011). Products approved for the preharvest treatment include imidacloprid (Marathon 1G or Marathon 60 WP), imidacloprid + cyfluthrin (Discus), or thiamethoxam (Flagship 0.22G or Flagship 25 WG) (NPB 2011). The preharvest treatments are less labor intensive and hazardous to apply than the immersion treatment (Oliver et al. 2007). However, preharvest treatments must be applied during summer months when susceptible first and second instars are present, several months in advance of fall to early spring harvesting (Mannion et al. 2001, Oliver et al. 2009). Subsequently, if producers are unable to sell their plants, the summer preharvest treatments will have been unnecessary and costly. To reduce cost to growers and also ensure availability of efficacious products, it is important to have alternative products available for use in the DJHP. Generic imidacloprid products have entered the market since the imidacloprid patent expired in 2006. Other insecticide chemistries may also be effective for control of Japanese beetle larvae in nursery settings with appropriate label amendments (e.g., chlorantraniliprole, clothianidin) (Koppenhöfer and Fuzy 2008, Morales–Rodriguez and Peck 2009). Testing generic and alternative insecticide chemistries is required to support their inclusion in the DJHP. The objectives of this study were to compare Japanese beetle control using standard DJHP insecticides (e.g., Discus SC, Marathon 60 WP, Flagship 0.22G) with non–DJHP-approved insecticides (i.e., Acelepryn Insecticide, Arena 50WDG, Allectus SC) and to evaluate efficacy of several generic imidacloprid products (i.e., Lada 2 F Insecticide, Mallet 2 F T&O Insecticide, Quali-Pro Imidacloprid 2 F T&O Insecticide; hereafter referred to as Discus, Marathon, Flagship, Acelepryn, Arena, Allectus, Lada, Mallet, and Quali-Pro, respectively).

Materials and Methods

Insecticide Treatments and Test Sampling Procedures. Multiple insecticides were evaluated against Japanese beetle larvae from 2007 to 2012 at commercial nurseries in central Tennessee producing balled and burlapped (B&B) trees in field soil (Table 1). All tests were performed using a randomized complete block design, with individual trees serving as the experimental unit. All tests had eight replications per treatment with the exception of the 2012 test, which had 10 replications.

Insecticide treatments were applied at labeled broadcast rate in May, June, July, or August to a 0.81-m^2 area at the base of each test tree. The treatment area was determined by the width of the harvested root ball (60 cm) plus an additional 15 cm past the edge of the root ball as required by the DJHP (NPB 2011). Insecticide sprays were applied using a handheld spray wand with a single TeeJet 8001VS (June, July, and August 2008) or TeeJet 8002VS (all other dates) flat fan nozzle (Spraying Systems Co., Wheaton, IL). Spray nozzles were held $\approx$45 cm above the soil surface and attached to a carbon dioxide backpack sprayer operating at $\approx$1.76 kg/cm^2 (25 psi). The sprayer was timed to deliver a spray volume of 83.7 ml/m^2 as required by the DJHP (NPB 2011). Water used to apply insecticide sprays had a pH of $\approx$6.5. No

Table 1. Insecticide trade products, active ingredient names, and field rates used in preharvest nursery tree sprays to control larval Japanese beetles

Trade name	AI	Rate/acre (lb AI)	kilograms AI/ha	Manufacturer
Acelepryn Insecticide	Chlorantraniliprole	0.21	0.23	DuPont Professional Products, Wilmington, DE
Allectus SC	Imidacloprid and Bifenthrin	0.25 + 0.20	0.28 + 0.22	Bayer Environmental Science, Research Triangle Park, NC
Arena 50WDG	Clothianidin	0.40	0.45	Valent U.S.A., Walnut Creek, CA
Discus SC	Imidacloprid and Cyfluthrin	0.50 + 0.12	0.56 + 0.13	OHP, Mainland, PA
Flagship 0.22G	Thiamethoxam	0.26	0.29	Syngenta Crop Protection, Greensboro, NC
Lada 2F Insecticide	Imidacloprid	0.40	0.45	Rotam North America, Miama, FL
Mallet 2F T&O Insecticide	Imidacloprid	0.40	0.45	Nufarm Turf and Specialty, Burr Ridge, IL
Marathon 60WP	Imidacloprid	0.40	0.45	OHP, Mainland, PA
Quali-Pro Imidacloprid 2F[a]	Imidacloprid	0.40	0.45	FarmSaver, Raleigh, NC

All sprays were applied at the labeled broadcast rate in a spray vol. of 83.7 ml/m^2 (87 gal/acre).
[a] Complete name is Quali-Pro Imidacloprid 2F T&O insecticide.

posttreatment irrigation was applied unless otherwise stated. Plots were mowed before applying insecticides if vegetation exceeded 7.6 cm as mandated by the DJHP (NPB 2011). Most test sites had a mix of grass and weed vegetation (>50% ground cover), as well as some bare soil.

All tests were evaluated for Japanese beetle survival in mid-October by digging the trees with a CareTree Systems model 501 tree spade (CareTree Systems, Columbus, OH). Root balls had top and bottom diameters of ≈60 and 30 cm, respectively, and a ball height of ≈50 cm. Root balls were placed on plywood, and the upper 35 cm of soil removed with a shovel. Soil was then examined using gardening tools and hands for scarab or curculionid larvae. Non-Japanese beetle scarab larvae were identified at least to genus level using a 16× hand lens to view the raster pattern (Shetlar and Andon 2012).

2007 Test. The test was conducted in a block of ash trees (*Fraxinus americana* L. variety 'Autumn Purple' or *Fraxinus pennsylvanica* Marshall variety 'Cimmaron'; ≈5 cm trunk diameter) at a commercial nursery in central Warren County, TN (35° 44′29.24″ N, 85° 47′50.10″ W). Trees were growing in Christian silt loam soil (fine, mixed, semiactive, mesic Typic Hapludults [well-drained, moderately slow to very slow permeability, slightly to extremely acidic]) (U.S. Dep. Agric.–
Natural Resources Conservation Service [USDA–NRCS] 2013). Insecticide treatments were applied on 24 May, 21 June, 23 July, or 22 August (Tables 2–4). To increase Japanese beetle oviposition under test trees,

Table 2. Mean (±SEM) and total *P. japonica* larvae recovered from field-grown nursery plants treated on the soil surface with insecticides at labeled rates and then harvested as 60-cm root balls during Oct. in multiple years

Insecticide	Timing	Live *P. japonica* larvae per root ball									
		2007 test		2008 test		2009 test		2010 test		2012 test	
		Mean ± SEM[a]	TL	Mean ± SEM	TL	Mean ± SEM	TL	Mean ± SEM	TL	Mean ± SEM	TL
Acelepryn	May	0.00 ± 0.00a[b]	0	0.25 ± 0.25a	2	0.00 ± 0.00a	0				
	June	0.00 ± 0.00a	0	0.38 ± 0.38a	3	0.00 ± 0.00a	0				
	July	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.50 ± 0.38a	4				
	Aug.	0.75 ± 0.41a	6	0.38 ± 0.18a	3	0.38 ± 0.26a	3				
Arena	May	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0		
	June	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0		
	July	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0		
	Aug.	0.13 ± 0.13a	1	0.13 ± 0.13a	1	0.00 ± 0.00a	0	0.00 ± 0.00a	0		
Allectus	July	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.50 ± 0.50ab	4		
Discus	July	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0
Marathon	July							0.00 ± 0.00a	0	0.00 ± 0.00a	0
Quali-Pro	July							0.00 ± 0.00a	0	0.00 ± 0.00a	0
Mallet	July							0.00 ± 0.00a	0	0.00 ± 0.00a	0
Lada	July									0.00 ± 0.00a	0
Flagship	July									0.00 ± 0.00a	0
Control		0.00 ± 0.00a	0	1.13 ± 0.61b	9	0.63 ± 0.32a	5	1.00 ± 0.42b	8	4.80 ± 0.87b	48

Insecticide treatments were compared by generalized linear interactive model with a log link assuming a negative binomial distribution with means separated by least squares means (df = 1 for each contrast). Test nursery trees were ash (2007 and 2008), dogwood (2009), and redbud (2010 and 2012). All tests were arranged in a randomized complete block design with 8 (2007–2010) or 10 (2012) replications. Treatments did not receive posttreatment irrigation; with the exception that 1.9 liters of water was applied to each replicate of the Aug. treatments in the 2008 test owing to excessively dry conditions, and 3.8 liters of water was applied to each replicate of the Flagship granular treatment in the 2012 test.
[a] SEM, standard error of mean; TL, total live *P. japonica* larvae collected for a given treatment.
[b] Means within a column followed by a different letter were significantly different ($P < 0.05$).

Table 3. Mean (±SEM) and total *Phyllophaga* spp. larvae recovered from field-grown nursery plants treated on the soil surface with insecticides at labeled rates and then harvested as 60-cm root balls during Oct. in multiple years

Insecticide	Timing	Live *Phyllophaga* spp. larvae per root ball									
		2007 test		2008 test		2009 test		2010 test		2012 test	
		Mean ± SEM[a]	TL	Mean ± SEM	TL	Mean ± SEM	TL	Mean ± SEM	TL	Mean ± SEM	TL
Acelepryn	May	0.25 ± 0.16a[b]	2	1.25 ± 0.49a	10	1.00 ± 0.50bc	8				
	June	0.25 ± 0.16a	2	0.88 ± 0.35a	7	0.25 ± 0.16ab	2				
	July	0.38 ± 0.38a	3	1.38 ± 0.75a	11	0.13 ± 0.13a	1				
	Aug.	1.38 ± 0.65bc	11	0.50 ± 0.27a	4	1.25 ± 0.62c	10				
Arena	May	0.00 ± 0.00a	0	0.75 ± 0.49a	6	0.13 ± 0.13a	1	0.38 ± 0.38a	3		
	June	0.13 ± 0.13a	1	0.88 ± 0.35a	7	0.50 ± 0.27a–c	4	0.38 ± 0.38a	3		
	July	0.50 ± 0.27ab	4	1.38 ± 1.10a	11	0.13 ± 0.13a	1	0.00 ± 0.00a	0		
	Aug.	0.63 ± 0.38ab	5	0.88 ± 0.40a	7	0.38 ± 0.26a–c	3	0.38 ± 0.26a	3		
Allectus	July	0.00 ± 0.00a	0	1.13 ± 0.35a	9	0.75 ± 0.31a–c	6	0.50 ± 0.38a	4		
Discus	July	0.63 ± 0.32ab	5	1.25 ± 0.62a	10	0.13 ± 0.13a	1	0.13 ± 0.13a	1	0.30 ± 0.21a	3
Marathon	July							0.25 ± 0.16a	2	1.50 ± 0.48cd	15
Quali-Pro	July							0.13 ± 0.13a	1	0.40 ± 0.22ab	4
Mallet	July							0.63 ± 0.26a	5	0.60 ± 0.34a–c	6
Lada	July									1.20 ± 0.53b–d	12
Flagship	July									0.90 ± 0.50a–d	9
Control		2.38 ± 0.75c	19	0.88 ± 0.48a	7	0.38 ± 0.18a–c	3	0.75 ± 0.37a	6	1.70 ± 0.40d	17

Insecticide treatments were compared by generalized linear interactive model with a log link assuming a negative binomial distribution with means separated by least squares means (df = 1 for each contrast). Test nursery trees were ash (2007 and 2008), dogwood (2009), and redbud (2010 and 2012). All tests were arranged in a randomized complete block design with 8 (2007–2010) or 10 (2012) replications. Treatments did not receive posttreatment irrigation; with the exception that 1.9 liters of water was applied to each replicate of the Aug. treatments in the 2008 test owing to excessively dry conditions, and 3.8 liters of water was applied to each replicate of the Flagship granular treatment in the 2012 test.

[a] SEM, standard error of mean; TL, total live *Phyllophaga* larvae collected for a given treatment.

[b] Means within a column followed by a different letter were significantly different ($P < 0.05$).

each tree received a phenethyl propionate: eugenol: geraniol (3:7:3) (PEG) bait on 20 June 2007, which is known to attract adult Japanese beetles (Jackson and Klein 2006). To make baits, a PEG-dispensing wafer (mini-con high void polyethylene wick porous-poly plastic GP-202; Genpore, Reading, PA) was cut into four equal parts. Wafer quarters were soaked in PEG until saturated and then placed inside a 4-cm-diameter by 1.5-cm-tall polyethylene plastic container (Cal Containers, Van Nuys, CA) with the lid closed. Three 0.64-cm-diameter holes were drilled in the lid to allow the PEG volatiles to escape. A plastic container with

Table 4. Mean (±SEM) and total curculionid larvae recovered from field-grown nursery plants treated on the soil surface with insecticides at labeled rates and then harvested as 60-cm root balls during Oct. in multiple years

Insecticide	Timing	Live curculionid spp. larvae per root ball									
		2007 test		2008 test		2009 test		2010 test		2012 test	
		Mean ± SEM[a]	TL	Mean ± SEM	TL	Mean ± SEM	TL	Mean ± SEM	TL	Mean ± SEM	TL
Acelepryn	May	0.13 ± 0.13a[b]	1	0.38 ± 0.26a	3	1.13 ± 0.52b–e	9				
	June	0.25 ± 0.16a	2	0.13 ± 0.13a	1	1.38 ± 0.71c–e	11				
	July	0.13 ± 0.13a	1	0.13 ± 0.13a	1	0.63 ± 0.32a–d	5				
	Aug.	0.25 ± 0.16a	2	0.25 ± 0.16a	2	2.00 ± 0.91e	16				
Arena	May	0.13 ± 0.13a	1	0.00 ± 0.00a	0	0.13 ± 0.13a	1	0.00 ± 0.00a	0		
	June	0.38 ± 0.26ab	3	0.13 ± 0.13a	1	0.13 ± 0.13a	1	0.13 ± 0.13a	1		
	July	0.63 ± 0.50ab	5	0.13 ± 0.13a	1	0.50 ± 0.38a–c	4	0.13 ± 0.13a	1		
	Aug.	0.00 ± 0.00a	0	0.38 ± 0.18a	3	0.38 ± 0.26ab	3	0.00 ± 0.00a	0		
Allectus	July	0.13 ± 0.13a	1	0.50 ± 0.19a	4	1.25 ± 0.41b–e	10	0.50 ± 0.19a	4		
Discus	July	0.88 ± 0.40ab	7	0.38 ± 0.26a	3	0.38 ± 0.18ab	3	0.25 ± 0.16a	2	0.80 ± 0.20a–c	8
Marathon	July							0.13 ± 0.13a	1	0.70 ± 0.30ab	7
Quali-Pro	July							0.38 ± 0.18a	3	1.30 ± 0.58bc	13
Mallet	July							0.38 ± 0.18a	3	0.50 ± 0.22ab	5
Lada	July									1.00 ± 0.33a–c	10
Flagship	July									0.30 ± 0.21a	3
Control		1.25 ± 0.62b	10	0.63 ± 0.38a	5	1.25 ± 0.56de	10	0.25 ± 0.16a	2	1.80 ± 0.36c	18

Insecticide treatments were compared by generalized linear interactive model with a log link assuming a negative binomial distribution with means separated by least squares means (df = 1 for each contrast). Test nursery trees were ash (2007 and 2008), dogwood (2009), and redbud (2010 and 2012). All tests were arranged in a randomized complete block design with 8 (2007–2010) or 10 (2012) replications. Treatments did not receive posttreatment irrigation; with the exception that 1.9 liters of water was applied to each replicate of the Aug. treatments in the 2008 test owing to excessively dry conditions, and 3.8 liters of water was applied to each replicate of the Flagship granular treatment in the 2012 test.

[a] SEM, standard error of mean; TL, total live curculionid larvae collected for a given treatment.

[b] Means within a column followed by a different letter were significantly different ($P < 0.05$).

a bait wafer was then attached to the trunk of each experimental tree with a plastic zip tie ≈15 cm above the ground. On 31 July 2007, an additional 0.5 ml of PEG was pipette-applied to each bait to refresh the lure. Trees were harvested for evaluation of larval survival on 9–11 October 2007.

2008 Test. The test was conducted at the same commercial nursery as the 2007 test using the remaining portion of untreated ash trees in the 2007 block. Soil type was the same as the 2007 test. Insecticide treatments were applied on 18 May, 13 June, 17 July, or 14 August (Tables 2–4). To increase Japanese beetle oviposition, each tree received a PEG bait as previously described for the 2007 test. However, instead of initially soaking the pheromone-dispensing wafers in PEG lure, the wafers received 1 ml of pipette-applied PEG lure on 19 June 2008. To further supplement oviposition, infestation cages were placed at the base of each tree on 24 July 2008. Cages were made from the bottom metal collection canister of an Ellisco Japanese beetle trap (EPA No. 24067-1) (Ellisco, Philadelphia, PA). The bottom end of the canister was removed to create a cage with two open ends. The cage bottom was pushed ≈5 cm into the soil ≈15 cm from each tree trunk. The top cage opening was closed by wedging a 150 ml plastic beaker into the entrance. Each beaker had five 2-mm holes drilled in the bottom to allow rainwater to enter the cage. Adult beetles were collected in Japanese beetle traps (Trécé, Adair, OK) baited with PEG lures and then subsequently placed in the infestation cages. Cages were infested with a 40 and 20 ml volume of adult beetles on 24 July and 8 August (≈210 total adult beetles per cage with assumed male:female sex ratio of 50:50), respectively. Owing to dry conditions, on 26 June and 1 and 2 July, replicate 1, replicate 2, and replicates 3–8, respectively, were watered with Tree Gator irrigation bags (Spectrum Products, Youngsville, NC) that delivered 18.9 liters water per tree. On 23 and 28 July and 5 and 22 August, all replicates were watered as previously described. Trees sprayed with insecticides on 14 August also received 1.9 liters of water with a sprinkle can posttreatment owing to excessively dry conditions. Trees were harvested for evaluation of larval survival on 14–15 October 2008.

2009 Test. The test was conducted in a nursery in southwestern Warren County, TN (35° 34′51.90″ N, 85° 50′41.84″ W), in a block of dogwood trees (*Cornus* spp.; ≈5–7.5-cm trunk diameter). Trees were growing in Waynesboro loam soil (fine, kaolinitic, thermic Typic Paleudults [well-drained, moderately permeable, strongly acidic]) (USDA–NRCS 2013). Insecticide treatments were applied on 18 May, 15 June, 24 July, or 17 August (Tables 2–4). To increase Japanese beetle oviposition, each tree received a PEG bait as described for the 2007 test, with 1 ml volume of PEG per bait applied on 15 June 2009. To further supplement oviposition, infestation cages described under the 2008 test were added on 14 July 2009. All cages received a 10 and 50 ml volume of adult beetles on 15 July and 16–22 July (≈210 total adult beetles per cage), respectively. Cages were removed on 23 July and not reinstalled. Trees were harvested for evaluation of larval survival on 13–14 October 2009.

2010 Test. The test was conducted in a nursery in southeastern Warren County, TN (35° 35′31.85″ N, 85° 45′45.01″ W), in a block of redbud trees (*Cercis canadensis* L.; ≈5 cm trunk diameter). Trees were growing in either Allen loam soil (fine-loamy, siliceous, semiactive, thermic Typic Paleudults [well-drained, moderately permeable, slightly to strongly acidic]) or Huntington silt loam soil (fine-silty, mixed, active, mesic Fluventic Hapludolls [well-drained, moderately permeable, moderately acidic to moderately alkaline]) (USDA–NRCS 2013). Insecticide treatments were applied on 26 May, 21 June, 19 July, or 16 August (Tables 2–4). To increase oviposition, each tree received a PEG bait on 24 June 2010. Baits were similar to those described in the 2007 test, but differed in that the PEG-soaked wafers were stapled directly to the tree trunk. The treated wafer was protected from rain by an aluminum weigh boat with one side trimmed open and then stapled to the tree trunk over the bait. On 22 July 2010, the baits were recharged with pipette-applied PEG until saturated. On 22 July 2010, all trees also had a metal Japanese beetle trap canister cage placed near the tree base as previously described in the 2008 test. Each canister received periodic infestations of adult Japanese beetles to supplement oviposition in the field plots between 22 July and 20 August. Cages were infested so that replicates all received equivalent infestations on the same date. Trees were harvested for evaluation of larval survival on 12–15 October 2010.

2012 Test. The test was conducted at the same nursery as the 2010 test in another block of *C. canadensis* trees (≈5 cm trunk diameter). Trees were growing in an Allen loam soil (USDA–NRCS 2013). Insecticide treatments were applied on 25 July 2012 (Tables 2–4). The Flagship treatment was applied at a rate of 11.3 grams per tree (equivalent to 0.29 kg [AI]/ha) using a salt shaker to uniformly dispense over the treatment area. Flagship granules were posttreatment irrigated as recommended on the insecticide label with 3.8 liters water using a sprinkle can. The sprayed treatments were not posttreatment irrigated. In this particular test, it was noted that the first posttreatment rain event occurred 6 d after treatment (31 July).

To increase Japanese beetle oviposition under test trees, on 13 June, pheromone-dispensing wafers were stapled to tree trunks as previously described in the 2010 test. Unlike 2010, wafers had PEG lure pipette-applied to saturation rather than being soaked in PEG. Wafers were covered with weigh boats as previously described to protect from rain. On 14 June, all trees had a metal Japanese beetle trap canister cage installed as previously described in the 2008 test. Each canister cage received five female beetles determined using foretibial characters (Fleming 1972a) on 19 July. Canister cages were removed on 25 July, so that spray treatments could be applied. On 1 August, canister cages were reinstalled, and each canister received five female beetles. On 17 August, each canister cage received one female beetle. On 24 August, canister cages in replicates 1 through 8 received one female beetle. Replicates 9 and 10 were not infested on this date owing to insufficient beetles. Trees were harvested for evaluation of larval survival on 9–14 October 2012.

Statistical Analysis. Numbers of Japanese beetle larvae were compared among insecticide treatments using a generalized linear interactive model (GLIM) (PROC GENMOD) with a log link, and assuming a negative binomial distribution, and treatment means separated by least squares means (Agresti 2002, SAS Institute 2003). For treatments with no larvae, a value of 0.5 was used in one replicate because GLIMs using the negative binomial distribution do not perform well with zero values. Significant differences were determined at $P = 0.05$ level.

Results

Japanese Beetle. In the 2007 test, no statistical differences were detected in numbers of Japanese beetle larvae among treatments ($\chi^2 = 14.2$; df = 10; $P = 0.162$) (Table 2). No Japanese beetle larvae were recovered in the check treatment. However, one and six Japanese beetle larvae were recovered in the August treatments of Arena and Acelepryn, respectively, indicating these insecticides did not provide 100% larval control at the August timing.

In the 2008 test, there were differences in numbers of Japanese beetle larvae among treatments ($\chi^2 = 24.2$; df = 10; $P = 0.007$) (Table 2). All insecticide treatments reduced the density of Japanese beetle larvae greater than the nontreated check, which averaged 1.13 larvae per root ball. Most insecticide treatments provided 100% Japanese beetle control with the exceptions being Arena in August (one larva) and Acelepryn in May, June, and August with two, three, and three larvae, respectively.

In the 2009 test, no statistical differences were detected in the numbers of Japanese beetle larvae among treatments ($\chi^2 = 16.2$; df = 10; $P = 0.093$) (Table 2). With the exception of Acelepryn in July and August treatments, all insecticide treatments provided 100% Japanese beetle control. The nontreated check averaged 0.63 Japanese beetle larvae per root ball, a low number that likely contributed to the inability to detect statistical differences between treated and check treatments.

In the 2010 test, there were differences in the numbers of Japanese beetle larvae among treatments ($\chi^2 = 23.8$; df = 9; $P = 0.005$) (Table 2). All insecticide treatments, except Allectus, provided complete and significantly greater Japanese beetle control than the check. The check treatment averaged 1.0 larva per root ball, a relatively low number. The two generic imidacloprid formulations (Mallet and Quali-Pro) provided Japanese beetle control that was as effective as the approved DJHP standards (i.e., Discus or Marathon).

In the 2012 test, there were differences in the number of Japanese beetle larvae among treatments ($\chi^2 = 164.9$; df = 6; $P = 0.0001$) (Table 2). All insecticide treatments provided complete and statistically greater Japanese beetle control than the nontreated check, which averaged 4.8 larvae per root ball. The three generic imidacloprid formulations (Mallet, Quali-Pro, and Lada) provided Japanese beetle control that was as effective as the approved DJHP standards (i.e., Discus, Marathon, or Flagship).

Other Scarab and Curculionid Larvae. In the 2007 test, other larvae recovered in the study included *Phyllophaga* spp. ($n = 52$) and unidentified curculionid larvae ($n = 33$). There were differences among insecticide treatments for *Phyllophaga* ($\chi^2 = 52.0$; df = 10; $P = 0.0001$) (Table 3) and curculionid ($\chi^2 = 26.2$; df = 10; $P = 0.004$) larvae (Table 4). All insecticides, with the exception of Acelepryn in August, had fewer *Phyllophaga* than the check treatment. Most treatments had significantly fewer curculionid larvae than the nontreated check; the exceptions being the June and July Arena and July Discus treatments.

In the 2008 test, other larvae recovered in the study included *Phyllophaga* spp. ($n = 89$), *Cotinis nitida* (L.) ($n = 4$), *Anomola* spp. ($n = 59$), and unidentified curculionid larvae ($n = 24$). No statistical differences were detected among treatments for *Phyllophaga* ($\chi^2 = 6.8$; df = 10; $P = 0.765$) (Table 3), *Anomola* ($\chi^2 = 8.8$; df = 10; $P = 0.552$) (data not shown), or curculionid ($\chi^2 = 9.8$; df = 10; $P = 0.458$) larvae (Table 4).

In the 2009 test, other larvae recovered in the study included *Phyllophaga* spp. ($n = 40$), *Cyclocephala* spp. ($n = 1$), and unidentified curculionids ($n = 73$). There were significant model differences among treatments for *Phyllophaga* ($\chi^2 = 24.6$; df = 10; $P = 0.006$), but no differences were detected between the insecticide treatments and the check (Table 3). There were differences among insecticide treatments for curculionids ($\chi^2 = 37.2$; df = 10; $P = 0.0001$) (Table 4), with all Arena timings and the Discus treatment having significantly fewer larvae recovered than the check treatment.

In the 2010 test, other larvae recovered in the study included *Phyllophaga* spp. ($n = 28$), *C. nitida* ($n = 3$), and unidentified curculionids ($n = 17$). No statistical differences were detected among treatments for *Phyllophaga* ($\chi^2 = 10.8$; df = 9; $P = 0.287$) (Table 3) or curculionids ($\chi^2 = 7.3$; df = 9; $P = 0.609$) (Table 4).

In the 2012 test, other larvae recovered in the study included *Phyllophaga* spp. ($n = 66$), *C. nitida* ($n = 11$), and unidentified curculionids ($n = 64$). There were differences among insecticide treatments for *Phyllophaga* ($\chi^2 = 19.8$; df = 6; $P = 0.003$) (Table 3) and curculionids ($\chi^2 = 16.7$; df = 6; $P = 0.01$) (Table 4). In terms of total larvae recovered, all insecticide treatments had fewer *Phyllophaga* and curculionids than the check treatment. However, for *Phyllophaga*, only Discus, Mallet, and Quali-Pro treatments had significantly fewer larvae than the check; for curculionids this was true for Marathon, Mallet, and Flagship.

Discussion

Most insecticide treatments applied at labeled rates reduced the numbers of Japanese beetle larvae when compared with the nontreated check. The imidacloprid- and thiamethoxam-based insecticides were only applied during July, while Acelepryn and Arena were applied May through August to evaluate other timings. The DJHP allows for preharvest banding of insecticides from May through July (NPB 2011). The May through July timing is intended to target first instars as a "preventative" treatment (Vittum et al. 1999, Mannion et al. 2001, Oliver et al. 2009). Treatments applied after July mostly target older second and third instars (Fleming 1972a), which are more difficult to kill and require insecticides with more "curative" properties (Vittum et al. 1999). Arena provided complete Japanese beetle larval control during May to July in four test years and also provided complete control in two out of four test years in August. In 2007 and 2008, one Japanese beetle larva was found in the August timing of Arena. The Nursery Accreditation Program (NAP) is the only procedure in the DJHP that gives a specific larval threshold for acceptable certification of nursery plants. Under the NAP, if more than one Japanese beetle larva is found in a nursery field while performing an acreage-based sampling protocol, accreditation is not granted. It is not possible to directly apply the sampling protocol of the NAP to the methods used in this study; however, if the NAP threshold of one Japanese beetle larva is used as a standard, then the single Japanese beetle larva found in the August timing of Arena may meet the criteria for category 2 states.

Japanese beetle larval control with Acelepryn was inconsistent for DJHP-level control. During 2008, Japanese beetle larvae were found at most of the application timings, and larvae were found in August at levels exceeding the one larvae NAP threshold in all test years. At the same Acelepryn rate used in this study, some irrigated turf trials also had Japanese beetle larval numbers (range, 0–0.8 larvae/ft^2) that would exceed DJHP thresholds during April to August timings (Heller et al. 2006a–c; Vittum et al. 2006; Heller and Kline 2007). Turf trials used smaller sampling areas and depths than our study, so it is likely larval numbers may have been even higher in the irrigated turf sites. Most August applications of Acelepryn also exceeded DJHP requirements in turf studies (Heller et al. 2006c, Smitley et al. 2006). Turf studies frequently use terminology like "adequate suppression" of white grub populations with Acelepryn, but for the DJHP where damage suppression is not the goal, Japanese beetle larval control needs to be near 100%. Acelepryn is currently labeled for use on sod farms, landscape ornamentals and turfgrass, and recreational turfgrass including golf courses, but is not labeled for use in commercial nurseries. Irrigation or rainfall is recommended on the Acelepryn label under the white grub section to wash the insecticide into the soil. Many field-grown nurseries operate dryland without irrigation, and treatments in our study were not posttreatment irrigated other than rainfall. However, comparing our Acelepryn results with turf studies, irrigation may still not have improved control to DJHP requirements.

The insecticides containing only imidacloprid as an active ingredient (i.e., Marathon, Lada, Mallet, and Quali-Pro) were 100% effective in every test year. Imidacloprid is rapidly toxic to newly eclosed first instar Japanese beetle, but has no effect on egg survival (George et al. 2007). Imidacloprid also has curative activity on second and third instar Japanese beetle (George et al. 2007), even though it is primarily recognized as a preventative type insecticide in the DJHP (NPB 2011). Discus, a combination product containing imidacloprid and cyfluthrin, was 100% effective in all five test years. Allectus, a combination product containing imidacloprid and bifenthrin, was 100% effective in three out of four test years, but exceeded the NAP threshold with four total Japanese beetle larvae recovered in the 2010 test. Allectus is labeled for use in residential turf and ornamentals and commercial and recreational areas, but not commercial nurseries. Allectus was included in the study to evaluate the benefit of using a combination product containing both imidacloprid and bifenthrin at rates lower than normally used for commercial nurseries. All of the other imidacloprid-based insecticides are labeled for nursery use. The labeled imidacloprid active ingredient rate for Discus (0.56 kg [AI]/ha) was higher than the rate for Allectus (0.28 kg [AI]/ha) or the other imidacloprid-based insecticides (0.45 kg [AI]/ha). The lower imidacloprid rate in Allectus may have contributed to the control failure in 2010. The pyrethroids, bifenthrin and cyfluthrin, are highly effective adulticides of Japanese beetle, and it is likely these products may have reduced adult egg-laying activity, as well as had some toxic effects on larvae (Baumler and Potter 2002). Allectus intoxicated female Japanese beetle and lowered egg-laying in some tests, but imidacloprid (Merit 75 WP) had no effect on adults (George et al. 2007). However, the Allectus effect on adult beetles was temporary and minimal in soils with older residues (George et al. 2007). It is likely most of the reduction in Japanese beetle numbers in Discus and Allectus treatments were because of imidacloprid effects on larvae and not adulticide effects of pyrethroids on egg-laying females. Generic imidacloprid insecticides (Lada, Mallet, and Quali-Pro) were just as effective as the current DJHP imidacloprid standards (Discus, Marathon), as well as the DJHP-approved thiamethoxam (Flagship). Generic imidacloprid products were all 2 F formulations, which are not currently approved for use in the DJHP (NPB 2011). The results indicate the 2 F formulation is adequate for DJHP usage. Flagship was only tested in 2012 and was 100% effective for controlling Japanese beetle.

All of the neonicotinoid products evaluated in this study, with the exception of Allectus during the 2010 test, provided 100% Japanese beetle control, which would be acceptable for DJHP standards for nursery shipments to category 2 states. However, neonicotinoids like imidacloprid and thiamethoxam do not always provide 100% Japanese beetle larval control. Mannion et al. (2001) found a range of 83–100% Japanese beetle control for imidacloprid products (Merit 75 WP, Marathon 1G, and Marathon 60WSP) and 0–100% for thiamethoxam products (CGA-293343 25

WG and 0.22G) during studies performed in field-grown nurseries from 1996 to 1999. At labeled rates, Oliver et al. (2009) found a range of 59–100% for imidacloprid products (Marathon 60 WP and Discus SC), 75–100% for thiamethoxam (Flagship 25 WG and 0.22G), 13–88% for dinotefuran (Safari 20SG), and 96–100% for clothianidin (Celero 16WSG). Marathon 1G and 60 WP, Discus SC, and Flagship 0.22G and 25 WG are approved for use in the DJHP for field-grown nursery stock shipments to category 2 states, despite the possible presence of Japanese beetle larvae in some of these treatments on occasion. Arena was very effective at controlling Japanese beetle from May to August in this study. August timings of neonicotinoids have not been consistent in other studies (Mannion et al. 2001, Smitley et al. 2006, Oliver et al. 2009), likely owing to greater numbers of second and third instars, which are less sensitive to insecticides (Vittum et al. 1999). Like Arena, another clothianidin product (Celero) was the most effective neonicotinoid tested in August in one other study (Oliver et al. 2009). It appears Arena may have potential as both an early season (May to July) preventative insecticide and a late season (August) curative insecticide.

Japanese beetle larval control in this study was evaluated under nursery production conditions, which means low densities of larvae. Smitley (1996) found that Japanese beetle larval numbers were fourfold fewer in nursery fields than adjacent grassy area, and 10-fold more abundant in weedy nursery fields (defined as >50% ground cover). All of the nursery blocks used in this study were not being managed with herbicides and had weed and grass vegetation at >50% ground cover. Other studies also report that weed or grass vegetation is preferred for egg-laying by Japanese beetle over bare soil sites (Szendrei and Issacs 2006). The low numbers of Japanese beetle larvae during some test years were likely owing to drought conditions and high summer temperatures, which are known to reduce egg-laying activity and larval survival (Fleming 1972a, Allsopp et al. 1992, Potter and Held 2002). In most tests, drought conditions occurred during the summer months with 2008 necessitating some irrigation to improve oviposition activity and larval survival. Dalthorp et al. (2000) concluded that moisture-related mortality in July–August was an important determinant of fall larval populations in a golf course fairway. Nursery sites with nonpreferred adult Japanese beetle hosts (i.e., ash, dogwood, and redbud) were selected for this study because egg-laying activity appears to be greater in nonhost than preferred host nursery blocks (Oliver et al. 2009). Other researchers have suggested a weak relationship between adult feeding damage on host trees or adult beetle populations in the area and subsequent grub densities in the fall (Dalthorp et al. 2000). Data reported by Szendrei and Issacs (2006) suggest adult beetles have different feeding and oviposition sites, which supports usage of sites with nonpreferred host trees. Smitley (1996) found areas with higher adult Japanese beetle trap captures also had higher larval densities in nursery blocks, which could support the benefit of the adult baiting technique used in this study to lure ovipositing females into the nursery blocks. All of the soils in this study were clay loams with low organic matter, which are preferred for oviposition by adult Japanese beetle (Allsopp et al. 1992, Dalthorp et al. 2000).

Insecticide treatments evaluated in this study reduced *Phyllophaga* spp. and curculionid larval numbers relative to the nontreated check in three out of the four test years, but, none of them eliminated these potential pests. Other scarabs like *C. nitida* and *Cyclocephala* spp. were also present, but in numbers too low to make conclusions about treatment effects. The 2008 test had a large number of *Anomola* spp. larvae, but no differences were detected between treatments. Scarab species vary considerably in larval susceptibility to imidacloprid and thiamethoxam, as well as the application timings of these insecticides (Vittum and Luce 2002). During the 2012 test year, the Flagship treatment had the lowest total curculionid recovery, which is consistent with other studies (Oliver et al. 2009) and suggests this compound may have activity on curculionids.

It is unlikely the treatments evaluated in this study will prevent the movement of these other Coleopterous larvae with nursery plant shipments. Unlike Japanese beetle, larvae of *Phyllophaga* species routinely cause significant damage to the root systems of trees (Luginbill and Painter 1953; Kard and Hain 1987, 1988). *Phyllophaga* larvae recovered in this study were often directly beneath the tree in the area with primary anchor roots, which may indicate feeding on nursery tree roots. Larvae were also sometimes at depths >15 cm. Most *Phyllophaga* species have a 2-yr life cycle in the southern states and 3-yr in the north (Luginbill and Painter 1953), which increases the likelihood of finding larvae deeper in the soil profile. Smitley (1996) did not find many *Phyllophaga* larvae during scarab surveys of Ohio and Michigan nurseries, but sampling with a cup cutter only allowed a soil depth of 15 cm and limited sampling in the root zone beneath the tree. Whole tree removal with sampling to 35 cm depth likely detected more *Phyllophaga* larvae in the soil around nursery plants than possible with surface sampling techniques. The phytosanitary significance of moving *Cyclocephala* and *Cotinis* larvae in nursery stock may be low because there are only a few species in these genera that are common in the eastern and midwestern United States (Vittum et al. 1999). In contrast, there are ≈220 described *Phyllophaga* species in the United States (Evans and Smith 2009). Luginbill and Painter (1953) detailed 40 out of 130 *Phyllophaga* species that occur in Tennessee. Four additional *Phyllophaga* species (*P. aemula* [Horn], *P. calceata* [LeConte], *P. forbesi* Glasgow, and *P. prununculina* [Burmeister]) also are likely to occur in Tennessee based on range distributions in adjacent states (Forschler and Gardner 1990). Thirteen *Phyllophaga* species found in Tennessee are considered destructive pests of field crops or turf, including *P. anxia* (LeConte), *P. crassissima* (Blanchard), *P. crenulata* (Froelich), *P. drakii* (Kirby), *P. ephilida ephilida* (Say), *P. fervida* (Fab.), *P. fusca* (Froelich), *P. futilis* (Le-

Conte), *P. gracilis gracilis* (Burmeister), *P. hirticula hirticula* (Knoch), *P. inversa* (Horn), *P. rugosa* (Melsheimer), and *P. tristis* (Fab.) (Luginbill and Painter 1953, Vittum et al. 1999). However, all of these pestiferous *Phyllophaga* species have wide spread distributions in the eastern and midwestern United States, so their nursery phytosanitary significance may be low. Many of these *Phyllophaga* species could be serious threats to agricultural systems west of the Rockies, where they are not endemic or likely to spread naturally, but category 1 state exclusion of field-grown nursery stock reduces the potential for accidental movement into these states. There are a number of pest curculionid species that damage nursery crops in genera such as *Otiorhynchus* and *Naupactus*, but it was unknown what species of curculionids were recovered in this study. At the present time, no scarabs other than Japanese beetle are under USDA regulatory program management, and the only weevil species under management are palm weevils (*Rhynchophorus* spp.) and the boll weevil (*Anthonomus grandis grandis* Boheman) (USDA–APHIS 2013), which suggests low USDA phytosanitary concern with regard to moving plants within eastern and midwestern regions that contain mostly endemic species of beetle larvae with extensive United States distributions.

In summary, all neonicotinoids evaluated in this study performed very well against larval Japanese beetle when applied in nonirrigated nurseries during July, as well as August in the case of Arena. Generic imidacloprid 2 F formulations were adequate for DJHP usage based on the results. Acelepryn did not perform at *P. japonica* control levels acceptable to the DJHP in this study, which may limit use of this chemical in the DJHP even with efficacy-enhancing factors like irrigation. If efficacy issues can be resolved, Acelepryn could provide an alternative mode of action (i.e., group 28 ryanodine receptor modulator) (Insecticide Resistance Action Committee [IRAC] 2012) to reduce the risk of insecticide resistance in Japanese beetle populations. Japanese beetle insecticide resistance has occurred in the past with usage of long residual insecticides (Niemczyk 1975, Lawrence et al. 1977, Ng and Ahmad 1979). Insecticide products with multiple modes of action would also provide more options to nursery producers in the event of regulatory removal of any of these insecticides. A label modification to include commercial nursery sites will be needed to use Arena, Allectus, or Acelepryn in the DJHP. Based on the results of this study, generic 2 F imidacloprid formulations and Arena are possible candidates for use in the DJHP.

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